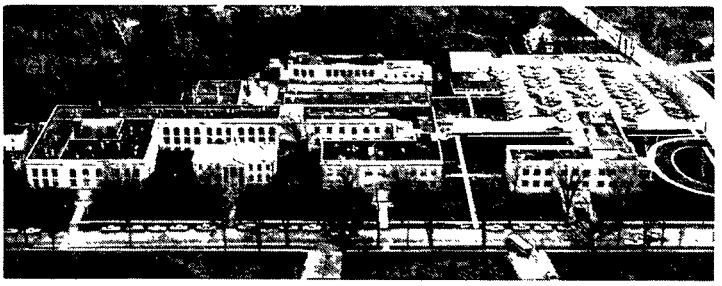

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THE ^{13}C–NMR SPECTRA OF THE XYLODEXTRINS AND THE CELLODEXTRINS

JOHN C. GAST, RAJAI H. ATALLA, AND
RONALD D. McKELVEY

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John C. Gast, Rajai H. Atalla, and Ronald D. McKelvey
The Institute of Paper Chemistry. Appleton, Wisconsin 54912 (U.S.A.)

ABSTRACT

The ^{13}C-NMR spectra have been recorded and assigned for the two homologous β-1,4-linked oligosaccharide series, the xylodextrins and the cellodextrins. The xylodextrins were studied through xylopentaose and the cellodextrins through cellotetraose. A spectrum of a low-DP cellulose in DMSO-d_6 was also assigned. In every case the spectra of the higher oligosaccharides closely parallel those of the corresponding disaccharides. Variations in line intensities made possible assignment of peaks to both terminal and internal units. A particularly important difference is observed between the chemical shifts at the internal C-4 for the two series of oligomers. It has been interpreted as evidence for significant differences in average linkage conformations which result from the absence of the 6-carbon in the xylodextrins.

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INTRODUCTION

Assignment of the ^{13}C-NMR spectra of various oligosaccharides has proven to be a useful method of evaluating the composition, configuration, and sequence of soluble polysaccharides[1-3]. The usual method of assignment is to compare the oligosaccharide spectrum to that of the constituent monosaccharides[4-5] or closely related disaccharides[2,6]. For example, it has been shown that the chemical shifts of the nonlinkage carbons of the internal saccharide units are not affected by chain length in the homologous α-1,4-linked maltodextrins[2].

In the case of homologous oligosaccharides it is also possible to use the variation of peak intensities with chain length to assist in the assignments[1]. The similarity of chemical shifts for equivalent carbons in the internal units can be used to distinguish peaks associated with them from those assigned to terminal units. Carbons several bonds removed from the linkage in the disaccharide should become relatively less intense in the oligosaccharide spectrum, while carbons close to the linkage will appear more intense. In this way lines of similar chemical shift can be differentiated on the basis of peak intensities, as this can be related to proximity to the glycosidic linkage.

In a study of factors that affect the β-1,4-linkage conformation of the xylo- and cellodextrins it was necessary to obtain and assign their ^{13}C-NMR spectra. The xylodextrins were studied through xylopentaose and the cellodextrins through cellotetraose. The spectra of these compounds have been considered on the basis of the corresponding mono- and disaccharides as well

as on the basis of variations in peak intensity. Intensity variations have been used to verify some of the previous assignments of cellobiose[5] in the case of the cellodextrins and to assist in developing assignments for the previously unassigned xylobiose*. Manifestation of differences in linkage conformation between the xylo- and cellodextrins have also been explored from these ^{13}C-NMR assignments.

RESULTS AND DISCUSSION

The spectral assignments for the cellodextrins in D_2O and a low-DP cellulose in DMSO-d_6 are given in Table I. In order to avoid contaminating our meager supply of the cellodextrins with a reference material we chose to set the C-1'+ of the nonreducing end-unit to the value of the equivalent cellobiose carbon. A close correspondence of peak locations is observed throughout the series from biose to tetraose permitting ready assignment of the triose and tetraose spectra. The assignments agree in general with those made by Inoue and Chujo[6] on cellodextrin fractions of DP 3.7 and 5.3; the exceptions being C-3 and C-5. Several different groups[8-10] have recently developed evidence to support reversal of the earlier assignments of C-3 and C-5. The new assignments have been adopted in the present study.

Examination of the assignments listed in Table I, as well as the line spectra in Fig. 1, reveals that differences from the cellobiose chemical shifts are 0.2 ppm or less for the equivalent carbons of the cellodextrins. The largest variations are at the carbon atoms, C-1, C-2, and C-4, on the internal units. These small changes may be a result of the influence of an additional substituent many bonds away (the next glucosyl ring) or the

*A paper being prepared develops xylobiose assignments from xylose, cellobiose, and various derivatives[7].

+The disaccharide nonreducing ring is primed.

effects of slight differences in linkage conformation or solvent hydration between the exterior and interior linkages. The influence of linkage conformation was suggested by Colson et al.[11] to explain the relatively large changes noted when the C-1 and C-4 chemical shifts of the maltodextrins were compared to those of the cyclodextrins.

[Table I and Figure 1 here]

The spectra shown in Fig. 1 also illustrate the differences in peak intensities which appear within the cellodextrin series. The variations in intensity are related to the location of a carbon, on the reducing end, the internal units, or the nonreducing end-unit. This follows from the equivalence or near equivalence of chemical shifts for carbons that occupy the same positions relative to the glycosidic linkage. The variation in environment is illustrated in Fig. 2 where the carbons in position 2 are taken as representative. In the disaccharide there are two types (a and b), while in the tetrasaccharide three types exist (a, b, and b'). However, those marked b and b' are essentially equivalent with respect to their position relative to a glycosidic linkage. The 2-carbon on the reducing ring, in contrast, has an environment which differs considerably. The intensities would approximate a 1:2:1 or 3:1 pattern depending on whether or not b and b' can be resolved. Assignments of peaks that are slightly shifted in the internal units are based on these variations in intensity.

[Figure 2 here]

The most downfield peak of cellobiose, the C-1' resonance at 103.4 ppm, is representative of the difference expected between b and b' in Fig. 2. In the spectra of the higher oligomers two peaks are observed in this region, at 103.2 and 103.4 ppm. Since the peak at 103.2 ppm increases in intensity relative to the 103.4 ppm peak, as the number of pyranose units increases, it

can be assigned to the internal linkage carbons (C-1) and the 103.4 ppm peak can be assigned to the nonreducing terminal unit. The spectrum of a low DP cellulose (Fig. 3) shows a small downfield shoulder corresponding to to the nonreducing end-unit C-1. Following a similar analysis the less intense of the pairs of lines centered at 73.9 and 79.4 ppm can be assigned to the 2-carbon and 4-carbon of oligomer terminal units, respectively.

[Figure 3 here]

In a reciprocal manner the spectra of the higher cellodextrins (Fig. 1) can be used to confirm some of the cellobiose assignments. An obvious example corresponding to the difference between a and b in Fig. 2 is the assignment of the 61.5 ppm resonance to the C-6 nonreducing end-group; this peak declines steadily in the spectra of the higher oligomers. The 61.0 ppm peak, which consists of the internal and reducing end-group 6-carbons, increases as expected in the higher cellodextrins. Thus, the differences in position of the 6-carbons, relative to the glycosidic linkage, appear to result in the changes observed in chemical shift.

A pattern of variation similar to the one reported above provides confirmation for the assignment of the peak at 75.1 ppm to C-2 in β-cellobiose. While previous authors[4-6] have reported two peaks in this region and viewed the 75.1 ppm peak as a coincidence of C-2 and either C-3 or C-5, in the present report three distinct peaks are resolved, the others appearing at 74.8 and 75.6 ppm. Since the 75.1 ppm peak declines steadily relative to the other two peaks with increasing chain length, the latter must contain contributions from carbons on internal units; differentiation of C-3 and C-5 is not possible on the basis of these spectra.

The spectrum of a low-$\overline{\overline{DP}}$ cellulose fraction in DMSO-d_6 is shown in Fig. 3. The appearance of the spectrum is similar to that reported by Gagnaire, Mancier, and Vincendon[10] for a cellulose of $\overline{\overline{DP}}$ 10. In the present work several reducing end-group peaks were also observed. The assignments for the low-$\overline{\overline{DP}}$ cellulose are consistent with those of the cellodextrins.

Previous reports[6] have suggested that the peak intensities of the C-1 peaks can be used to estimate the number average $\overline{\overline{DP}}$ for low molecular weight oligosaccharides. Comparison of these intensities for cellotriose, cellotetraose, and the low-DP cellulose suggest that this correlation is not generally true. The cellotriose spectrum, in particular, shows significant differences in the intensities for C-1 in the nonreducing and internal units suggesting that the two carbons possibly have different spin-lattice relaxation times.

The ^{13}C-NMR spectra of the xylodextrins are graphically represented in Fig. 4 with the corresponding assignments in Table II. Assignments for xylobiose have been developed from comparisons with assignments for xylose[8,12,13] and cellobiose. Again, as in the cellodextrins, intensity variations confirm these assignments. For example, changes in the intensities of the lines at 66.1, 63.9, and 59.8 ppm support assignment of the 63.9 ppm line to C-5 of β-xylobiose and the internal C-5 carbon resonances of the higher oligomers. Such an assignment is not obvious from comparisons with xylose, where, based on chemical shift only, the peak at 66.1 ppm would be assigned to C-5 in β-xylobiose.

[Figure 4 and Table II here]

In the case of the xylodextrins the chemical shifts are reported relative to the C-1 of the nonreducing unit of xylobiose. In comparing the series, xylobiose through xylopentaose, the shifts of carbons-2 and 5 do not vary by more than 0.2 ppm from the values anticipated from the xylobiose spectrum. Carbons-1 and 4 next to the glycosidic linkage vary to a slightly greater extent suggesting that slightly different conformations might exist at the interior linkages[2,11]. Internal C-3 carbons undergo upfield shifts of the same order of magnitude indicating the sensitivity to conformational change at the linkage. As in the case of the cellodextrins the small shifts can also arise from the substitution of a monosaccharide unit for a proton in the internal units.

The contrasts between xylodextrin and cellodextrin spectra suggests significant differences in the constraints on the linkage between the two series. While the chemical shift of C-4 differs slightly between glucose and xylose[17], indicating that substitution of a methylene hydroxyl (C-6) at C-5 in glucose has a relatively small effect, the difference between the linkage C-4 chemical shifts of the xylodextrins and cellodextrins is approximately 1.8 ppm in D_2O*. Such a large difference would not be expected on the basis of the effect of substitution of a xylosyl _vs._ a glucosyl unit[3]. It appears most likely that this difference is a manifestation of significant differences in the average conformations of the β-1,4-linkages in the two homologous dextrin series. Such an interpretation is consistent with model studies of the disaccharide linkage conformation[14]. Different average linkage conformations may explain differences in reactivity and solubility of the two types of oligosaccharides.

*In DMSO-d_6 the difference becomes 5.2 ppm.

Colson, et al.[11], in comparing the chemical shifts of the linkage C-1 and C-4 in maltotriose to those of the cyclodextrins, found a downfield shift of 1.8 and 4.0 ppm, respectively, for the more constrained cyclodextrins. The comparable shifts for the cellodextrins relative to the xylodextrins are downfield by 0.7 and 1.8 ppm.

The greater constraints at the linkage in the case of the cellodextrins appear to result from the presence of C-6. The role of C-6 is suggested by the pattern of chemical shifts of the disaccharides in Table III. The linkage C-4 chemical shifts of xylobiose and cellobiose, relative to the appropriate monosaccharides, are compared to several other β-1,4-linked disaccharides. Mannobiose and 4-O-β-D-glucosyl-D-mannose, both of which have a C-6 relative to the linkage analogous to that of cellobiose, have chemical shifts relative to the respective monosaccharides similar to that of cellobiose. In contrast, the C-4 chemical shifts for 4-O-β-D-galactosyl-D-xylose[16] and 4-O-β-D-glucosyl-D-xylose, which do not have a C-6 on the reducing ring, are nearly identical to that of xylobiose.

[Table III here]

SUMMARY

Internally consistent assignments have been developed for the ^{13}C-NMR spectra of the xylodextrins and the cellodextrins. Peak intensity variations with chain length made it possible to distinguish between the resonances of terminal and internal monosaccharide units. These intensity variations also permitted verification of previously published cellobiose assignments.

While differences between the chemical shifts of equivalent carbons on internal and terminal units are slight, larger changes are observed for the

linkage carbons C-1 and C-4. This may result from variations in linkage conformation along the chain. A more significant observation is the difference between the C-4 chemical shifts for xylobiose and cellobiose. Comparison with several disaccharide models suggests that the absence of the 6-carbon on the reducing-end allows a much wider range of conformations for the glycosidic linkage. Thus the β-1,4-linkage appears to be more flexible in the xylodextrins than in the cellodextrins.

EXPERIMENTAL

^{13}C-NMR spectra were recorded on a Joel FX-100 NMR spectrometer operating at 25.00 MHZ in the noise decoupled mode. Spectra were accumulated for 50,000-100,000 pulses for the trioses, tetraoses, mannose disaccharides and xylopentaose using a 5000 Hz spectral width and a 1 second pulse interval, and 8192 data points. All spectra were recorded at room temperature except cellotetraose (44°C) and low-DP cellulose (75°C). The Joel microprobe was used to obtain the spectra of the trioses, tetraoses, xylopentaose, mannobiose, and 4-O-β-D-glucosyl-D-mannose because of limited available sample. Concentrations of 2-8 mg in 75 μL of D_2O were used. The cellobiose, xylobiose, and 4-O-β-D-glucosyl-D-xylose spectra were recorded using a 5 mm probe at equivalent concentrations with internal p-dioxane as a reference. All other chemical shifts were referenced to the value of the internal anomeric carbon of either the relevant disaccharide or mannose. This was done to avoid potential contamination of the small amounts of available sample with any internal reference. The low-DP cellulose spectrum was recorded using a 10 mm probe with DMSO-d_6 as the solvent.

The xylodextrins were obtained from the collection of carbohydrates available at The Institute of Paper Chemistry as were the mannose disaccharides.

The cellodextrins were prepared by acid hydrolysis of cellulose followed by carbon-celite column chromatography[15]. The low-DP cellulose was obtained from the methanol treated filtrate of a phosphoric acid hydrolyzed Whatman CF-1 cellulose powder. Xylobiose and 4-O-β-D-glucosyl-D-xylose were synthesized; the synthetic sequence is being reported elsewhere[7]. Cellobiose was purchased (Matheson, Coleman, and Bell).

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Gorin, P. A. J., Can. J. Chem., 51 (1973) 2375-2383.

2. Friebolin, H., Frank, N., Keilich, G., and Siefert, E., Die Makromol. Chemie 177 (1976) 845-858.

3. Colson, P. and King, R. R., Carbohydr. Res. 47 (1976) 1-13.

4. Dorman, D. E., and Roberts, J. D., JACS 93 (1971) 4463-4472.

5. Usui, T., Yamaoka, N., Matsuda, K., Sugiyama, H., Seto, S., and Tuzimura, K., J. Chem. Soc. Perkins I (1973) 2425-2432.

6. Inoue, Y. and Chujo, R., Carbohydr. Res. 60 (1978) 367-370.

7. Gast, J. C., Kidd, J. R., and Schroeder, L. R., paper in process.

8. Gorin, P. A., and Mazurek, M., Can. J. Chem. 53 (1975) 1212-1223.

9. Balza, F., Cyr, N., Hamer, G. K., Perlin, A. S., Koch, H. J., and Stuart, R. S., Carbohydr. Res. 59 (1977) C7-C11.

10. Gagnaire, D., Mancier, D., and Vincendon, M., Paper presented at ACS National Meeting, Chicago, Aug. 29, 1977.

11. Colson, P., Jennings, H. J., and Smith, I. C. P., JACS 46 (1974) 8081-8086.

12. Dorman, D. E., and Roberts, J. D., JACS 92 (1970) 1355-1361.

13. Walker, T. E., London, R. E., Whaley, T. W., Barker, R., and Matwiyoff, N. A., JACS 98 (1976) 5807-5813.

14. Rees, D. A. and Skerrett, R. J., Carbohydr. Res. 7 (1968) 334-348.

15. Whistler, R. L. and Durso, D. F., JACS 72 (1950) 677-679.

16. Erbing, B., Lindberg, B., and Norberg, T., Acta Chem. Scand. B32 (1978) 308-310.

17. Stothers, J. B., Carbon-13 NMR for organic chemists (1972).

TABLE I

^{13}C-NMR CHEMICAL SHIFTS[a] OF THE CELLODEXTRINS IN D_2O SOLUTION

			C-1	C-2	C-3	C-4	C-5	C-6
Cellobiose	Reducing end-unit	α	92.6	72.2[b]	72.3[b]	79.7	70.9	61.0
		β	96.6	75.1	74.8[b]	79.5	75.6	61.1
	Nonreducing end-unit		103.4	74.0	76.4[b]	70.3	76.8	61.5
Cellotriose	Reducing end-unit	α	92.7	72.1	72.1[b]	79.5[c]	70.9[b]	60.8
		β	96.6	75.1	74.9[b]	79.5[c]	75.7[b]	60.8
	Internal units		103.2	73.8[c]	74.8[b]	79.3[c]	75.7[b]	60.8
	Nonreducing end-unit		103.4	74.0[c]	76.4[b]	70.4	76.8[b]	61.5
Cellotetraose	Reducing end-unit	α	92.7	72.2	72.2[b,d]	79.4[c,e]	71.0[b]	60.9
		β	96.6	75.2	75.0[b,d]	79.4[c,e]	75.7[b]	60.9
	Internal units		103.2	73.8[c]	75.0[b,d]	79.3[c,f]	75.7[b]	60.9
	Nonreducing end-unit		103.4	74.1[c]	76.5[b]	70.4	76.9[b]	61.6
	In DMSO-d_6							
Cellulose	Reducing end-unit	α	92.0	s[g]	s[b]	80.7[e]	s[b]	60.6
		β	96.8	s	74.9[b]	80.7[e]	75.1[b]	60.6
	Internal units		102.7	73.2	74.9[b]	80.1	75.1[b]	60.6
	Nonreducing end-unit		s		76.7	70.3	76.9	61.2

[a] Chemical shifts in ppm relative to TMS by setting the terminal nonreducing end-unit C-1 equal to the value observed in cellobiose. The cellobiose shifts are relative to dilute p-dioxane at 67.4. The cellulose is referenced to DMSO-d_6 at 39.5 ppm from TMS.

[b] Some authors reverse these assignments[4,5].

[c] Assignments confirmed by the low-DP cellulose spectrum.

[d] Resolved in cellotriose only.

[e] Observed as shoulders on the internal C-4 peak.

[f] Actually two distinct peaks are resolved.

[g] Peak observed as a weak shoulder on a larger peak or as a very weak peak are marked(s).

TABLE II

^{13}C-NMR CHEMICAL SHIFTS[a] OF THE XYLODEXTRINS IN D_2O SOLUTION

			C-1	C-2	C-3	C-4	C-5
Xylobiose	Reducing end-unit	α	92.8	72.3[b]	71.9[b]	77.5	59.8
		β	97.3	74.9[c]	74.9[c]	77.3	63.9
	Nonreducing end-unit		102.7	73.7	76.5	70.1	66.1
Xylotriose	Reducing end-unit	α	92.8	72.2[b]	71.8[b]	77.2	59.7
		β	97.3	74.8	74.8	77.2	63.8
	Internal units		102.5	73.6	74.5	77.2	63.8
	Nonreducing end-unit		102.7	73.6	76.5	70.0	66.1
Xylotetraose	Reducing end-unit	α	92.8	72.2[b]	71.8[b]	77.2	59.7
		β	97.3	74.7	74.7	77.2	63.8
	Internal units		102.5	73.5	74.5	77.2	63.8
	Nonreducing end-unit		102.7	73.5	76.4	70.0	66.1
Xylopentaose	Reducing end-unit	α	92.8	72.2[b]	71.8	77.2	59.7
		β	97.3	74.7	74.7	77.2	63.8
	Internal units		102.5	73.5	74.5	77.2	63.8
	Nonreducing end-unit		s[d]	73.5	76.4	70.0	66.1

[a] Chemical shifts in ppm relative to TMS by setting the terminal nonreducing C-1 equal to the value observed in xylobiose. The xylobiose spectrum is referenced to dilute p-dioxane given a value of 67.4 ppm.

[b] These resonances may be exchanged.

[c] These resonances are slightly resolved in xylobiose.

[d] A shoulder on the 102.5 ppm peak.

TABLE III

CHEMICAL SHIFTS OF THE LINKAGE C-4 FOR SEVERAL β-1,4-LINKED DISACCHARIDES

Compound	C-4 Chemical Shift	Chemical Shift Change Relative to Monosaccharide
β-Xylobiose	77.3	7.1
4-O-β-D-galactosyl-D-xylose	77.7[a]	7.5
4-O-β-D-glycosyl-D-xylose	77.4	7.2
β-Cellobiose	79.5	8.9
β-Mannobiose	77.5	9.9
4-O-β-D-glucosyl-β-D-mannose	77.3	9.7
β-Xylose	70.2[b]	
β-Glucose	70.6[b]	
β-Mannose	67.6[b]	

[a] Relative to external TMS[16].
[b] See Reference 17.

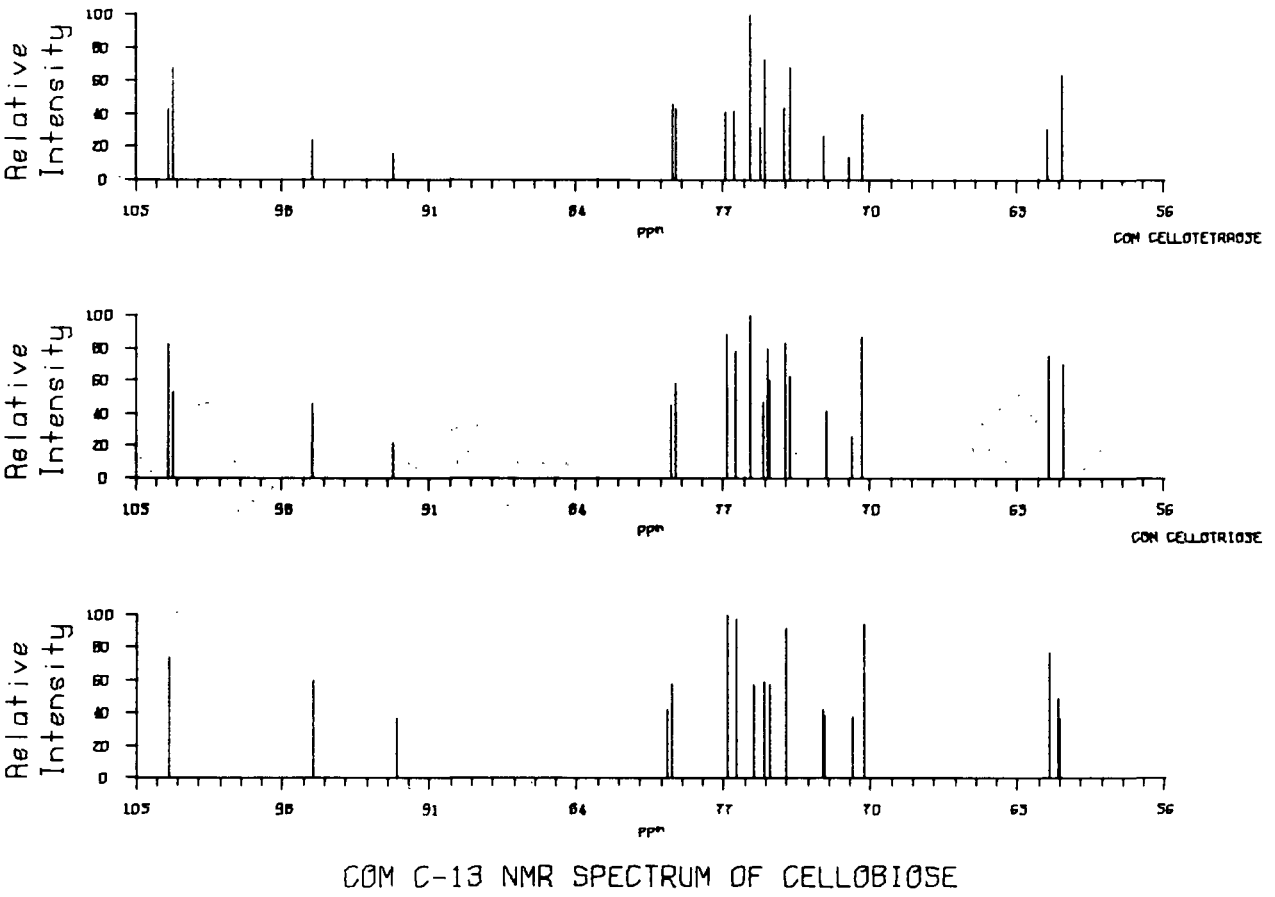

Figure 1. Comparison of Cellobiose Through Cellotetraose ^{13}C-NMR Spectra

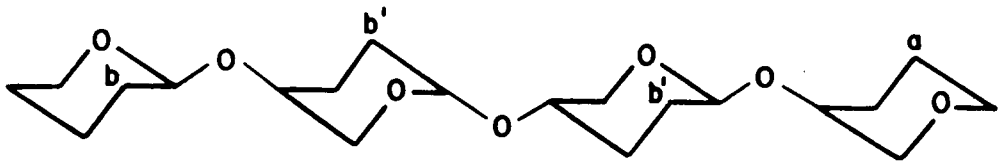

Figure 2. An Illustration of the Nearly Equivalent Carbons of a Homologous Oligosaccharide

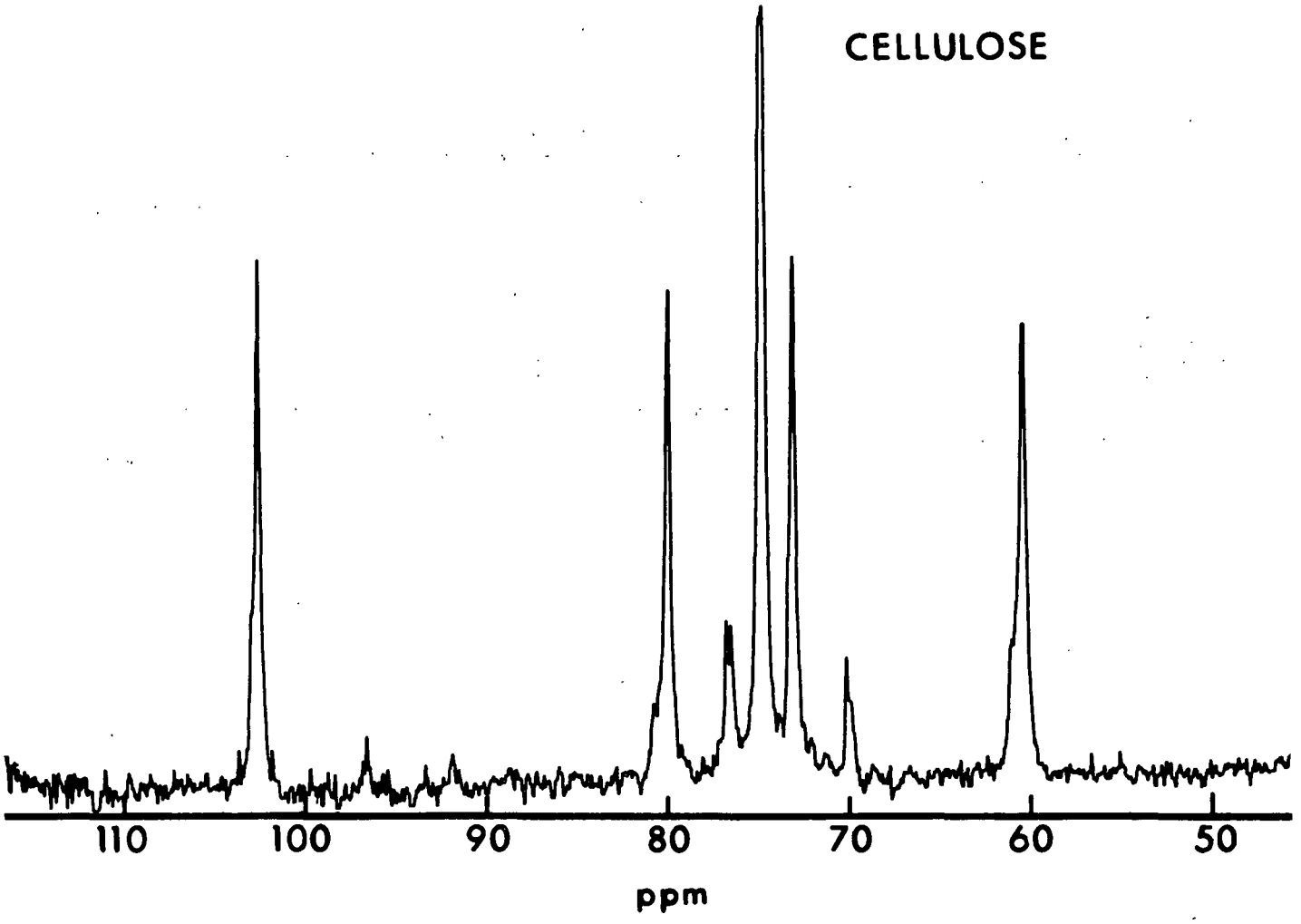

Figure 3. The ^{13}C-NMR Spectrum of a Low-DP Cellulose

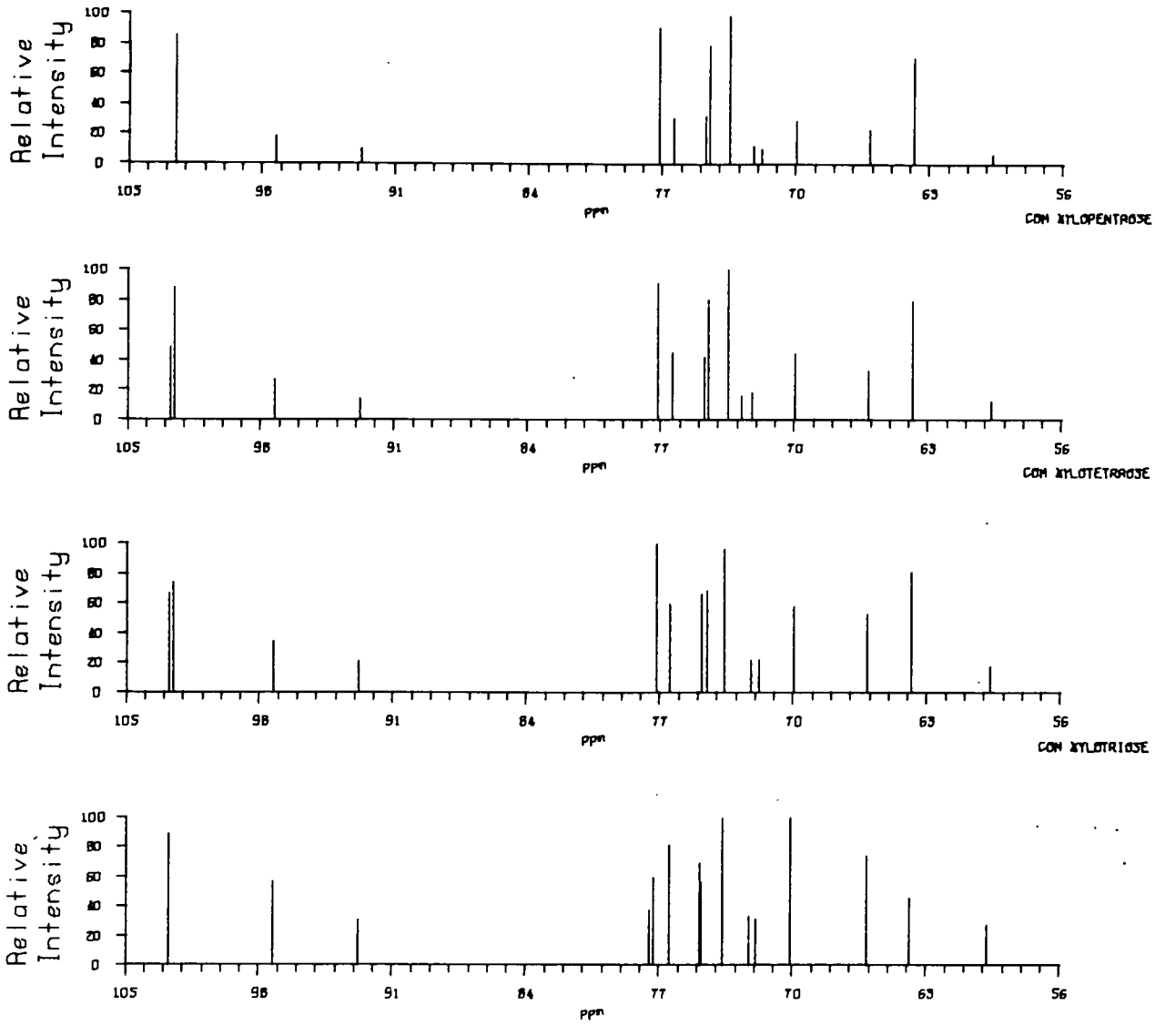

Figure 4. Comparison of the ^{13}C-NMR Spectra of Xylobiose Through Xylopentaose